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R. Senaratne · D.S. Ratnasinghe

Nitrogen fixation and beneficial effects of some grain legumes and green-manure crops on rice

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Abstract Studies were conducted on paddy soils to ascertain N_2 fixation, growth, and N supplying ability of some green-manure crops and grain legumes. In a 60-day pot trial, sunhemp (*Crotalaria juncia*) produced a significantly higher dry matter content and N yield than *Sesbania sesban, S. rostrata,* cowpeas (*Vigna unguiculata*), and blackgram (*V. mungo*), deriving 91% of its N content from the atmosphere. Dry matter production and N yield by the legumes were significantly correlated with the quantity of N_2 fixed. In a lowland field study involving sunhemp, blackgram, cowpeas, and mungbean, the former produced the highest stover yield and the stover N content, accumulating 160–250 kg N ha^{-1} in 60 days, and showed great promise as a biofertilizer for rice. The grain legumes showed good adaptability to rice-based cropping systems and produced a seed yield of 1125–2080 kg ha^{-1}, depending on the location, species, and cultivar. Significant inter- and intraspecific differences in the stover N content were evident among the grain legumes, with blackgram having the highest N (104–155 kg N ha^{-1}). In a trial on sequential cropping, the groundnut (*Arachis hypogaea*) showed a significantly higher N_2 fixation and residual N effect on the succeeding rice crop than cowpeas, blackgram, mungbeans (*V. radiata*), and pigeonpeas (*Cajanus cajan*). The growth and N yield of the rice crop were positively correlated with the quantity of N_2 fixed by the preceding legume crop.

Key words N_2 fixation · N supplying potential · Grain legumes · Green manuring · ^{15}N · Wetland rice

R. Senaratne (✉) · D.S. Ratnasinghe[1]
Department of Crop Science,
Faculty of Agriculture,
University of Ruhuna, Mapalana,
Kamburupitiya, Sri Lanka

Present address:
[1] School of Agriculture,
Department of Agriculture,
Angunukolapalassa, Sri Lanka

Introduction

High-yielding rice varieties developed following the green revolution have substantially increased global rice production. However, these varieties need heavy fertilizer inputs. The economic and environmental costs of the heavy use of chemical N fertilizers have seriously restricted the use of high-yielding varieties in rice culture, affecting production. Rice is cropped on about 145 million hectares in the world with a production of about 470 million tons (Roger and Ladha 1992). More than half of the world population is dependent on rice, and an additional 300 million tons of rice will be required annually by 2020 to meet the needs of the growing population. This will require a 65% production increase within 30 years without much expansion of the cultivated area (International Rice Research Institute 1989). The need for sustained rice cropping had led to an urgent search for alternatives to chemical N fertilizer. Hence, increasing attention is being paid to biological N_2 fixation to meet the N requirements of rice (George et al. 1992).

Aquatic legumes, such as *S. aculaeta, S. rostrata* and *Aeschynomene afraspera*, when grown as pre-rice green-manure crops, can add over 100 kg N ha^{-1} within 50–60 days (Roger and Watanabe 1986; Ladha et al. 1988). These green-manure crops can add substantially higher amounts of N than is required by rice (Ladha et al. 1988), with a fertilizer substitute value of 60–120 kg N ha^{-1} as urea (Becker et al. 1990). Grain legumes such as cowpeas, mungbeans, and blackgram are increasingly being planted in rice-based cropping systems as an opportunity/insurance crop when water is limiting for rice (Wood and Myers 1987). Besides producing an economic seed yield, these legumes can add considerable amounts of N to the soil (Kulkarni and Pandey 1988; Buresh and De Datta 1991). The present study was conducted to ascertain the N_2 fixation, adaptability, and N contribution of some green-manure crops and grain legumes when grown on paddy soils.

Materials and methods

Experiment 1

An experiment was conducted outdoors in pots (45×30 cm) from September to November 1991, at Hungama (6°15′ N, 80°54′ E, 5 m above mean sea level), in the Hambantota District, Sri Lanka. The pots were filled with a paddy soil (Reddish Brown Earth, classified as Rhodustalfs) at the rate of 17 kg pot^{-1} (Table 1). Each pot was supplied with N in the form of ^{15}N-labelled ammonium sulphate (ca. 5% enrichment), P, and K at 10, 25, and 25 mg kg^{-1} soil, respectively, and mixed thoroughly. Grain legumes and green-manure crops (Table 2) were grown, four to a pot, in a randomized complete block design with five replicates. Rice (cv. BW 300) was used as the reference crop to determine N_2 fixation by ^{15}N methodology. During the experimental period the plants were watered, weeded, and sprayed against pests and diseases as necessary. Sixty days after planting, the above-ground material was harvested, dried to a constant weight, and the dry weight recorded. The samples were then finely ground and per cent N (Bremner and Mulvaney 1982), atom per cent ^{15}N excess (Fiedler and Proksch 1975), and N_2 fixation (McAuliffe et al. 1958) were determined.

Experiment 2

This experiment was conducted at two locations, Hungama and Angunukolapalassa, from April to June, 1992. Three grain-legume crops and one green-manure crop (Table 4) were planted in farmers' paddy fields. The experimental plots measured 4×4 m and were arranged in a randomized complete block design with four replicates. The green-manure crop, sunhemp, was harvested 60 days after planting and the grain legumes were harvested 85 days after planting. Fallen leaves from the grain legumes were also collected at the harvest. The dry weight of stover, its per cent N, and the seed yield of the crops were determined as described in experiment 1.

Experiment 3

This experiment was conducted in pots (45×30 cm) outdoors from March to July, 1992, at Hungama, with the paddy soil used in experiment 1. There were two stages. In stage 1, N_2 fixation by five grain legumes (Table 5) was measured by ^{15}N methodology using maize as the reference crop. In stage 2, the effect of N_2 fixation on the growth and N yield of a succeeding rice crop was determined against maize (control). In stage 1 the effects of sesame (another non-legume), and a fallow on rice were also compared with those of maize. The treatments in stage 1 were replicated five times and arranged in a randomized complete block design in two sets.

After sowing the seeds, ^{15}N-labelled ammonium sulphate was applied to one set as described in experiment 1. The other set was fertilized with N at the same rate but in the unlabelled form. Four plants per pot were maintained. Cultural practices, including P and K application, were similar to those in experiment 1. At maturity, plants in the ^{15}N-labelled set were harvested, processed, and per cent N, atom per cent ^{15}N excess, and N_2 fixation were determined as described before. Simultaneously, the pods in the unlabelled set were harvested and the stover (residue) was incorporated into the soil and mixed thoroughly. Two weeks later rice was oversown in all pots (unlabelled) and fertilized with N, P, and K at 10, 15 and 25 mg kg^{-1} soil, respectively. At maturity, the crop was harvested and processed, and the dry weight and per cent N determined as described above. Correlation coefficients between N_2 fixed by the legumes and the dry matter production and N yield of the succeeding rice crop were determined.

Results

Experiment 1: N acquisition, growth, and yield of legumes

The per cent and quantity of N derived by the legumes from the soil and atmosphere are given in Table 2.

Table 1 Chemical properties of soils

Experiment	pH (H_2O)	Organic matter (%)	Olsen P (mg kg^{-1})	Exchangeable K (mEq 100 g^{-1})	Total N (%)	Ca (mEq 100 g^{-1})	Mg (mEq 100 g^{-1})
1 and 3	5.4	0.7	7.0	0.41	0.097	8.9	2.00
2 (at Hungama)	4.6	0.8	4.0	0.23	0.080	4.9	1.63

Table 2 N acquisition, growth, and N yield of grain legumes and green-manure crops 60 days after planting, experiment 1

Within a column, values followed by the same letter are not significantly different at $P<0.05$

Crop	Percentage of N derived from		Quantity of N derived from		Total dry matter (g pot^{-1})	Total N (g pot^{-1})
	Soil (%)	Atmosphere (%)	Soil (g pot^{-1})	Atmosphere (g pot^{-1})		
Sunhemp (*Crotalaria juncia*)	8.0d	90.7a	0.339a	4.054a	169.3a	4.45a
Sesbania sesban	41.9a	50.1c	0.228bc	0.284c	26.8c	0.57c
S. rostrata	16.1b	81.3b	0.157c	0.770bc	30.4c	0.94c
Cowpea cv. Bombay	14.4bc	83.3ab	0.244abc	1.510b	57.0b	1.77b
Cowpea cv. Arlington	14.0bc	83.9ab	0.221bc	1.396b	66.6b	1.85b
Cowpea cv. 889	16.9b	80.1b	0.312ab	1.500b	72.2b	1.87b
Blackgram cv. MI-1	12.3c	85.7ab	0.228bc	1.574b	57.3b	1.84b

Sunhemp, by far, showed the highest N_2-fixation potential, deriving 91% of its N content from the atmosphere, fixing 4 g N pot^{-1} (1591 cm^2), significantly more than all the other legumes. There was no significant difference in the quantity of N_2 fixed by the grain legumes, either between species or between cultivars. *S. Sesban* showed the lowest N_2 fixation, deriving only 50% of its N yield from the atmosphere. The growth of both *S. sesban* and *S. rostrata* was poor and produced the lowest dry matter production and N yield. There were no stem nodules on *S. rostrata*, which was not inoculated. As with N_2 fixation, sunhemp produced the highest phytomass and N yield, which were significantly higher than those from other legumes. Dry matter production and N yield by the legumes were significantly correlated with the per cent N derived from the atmosphere and the quantity of N_2 fixed (Table 3).

Table 3 Correlation coefficients between N_2 fixation and the growth of the legumes, experiment 1 (*Ndfa* N derived from atmosphere)

	Total dry matter	Total N content
% Ndfa	0.380*	0.450**
N_2 fixed	0.918**	0.987**

*$P<0.05$, **$P<0.01$

Experiment 2: Stover yield, N content in stover, and seed yield in legumes

The grain legumes were harvested 80 days after planting while sunhemp was harvested 60 days after planting. Sunhemp produced the highest total dry matter at one site and did not differ from blackgram at the other (Table 4). The stover yield and the stover N content were highest in sunhemp at both locations. Significant inter- and intraspecific differences in stover yield and stover N content were evident among the grain legumes, suggesting possible variation in the N residual value. Among the grain legumes used, blackgram was the most promising source of residual N at both locations. The seed yield of the grain legumes varied from 1125 to 2083 kg ha^{-1}, depending on the location, legume, and cultivar (Table 4). Thus, in general, the legumes showed good adaptability to rice-based cropping systems.

Experiment 3: Acquisition of N from the soil and atmosphere

There were significant differences among the legumes in the per cent and quantity of N derived from the soil and atmosphere (Table 5). Groundnuts showed the highest N_2 fixation among the five legumes followed by blackgram.

Table 4 Stover production, N content in stover, and seed yield of legumes, experiment 2

Crop	Hungama				Angunukolapalassa			
	Stover yield (kg ha^{-1})	Seed yield (kg ha^{-1})	Total dry matter (kg ha^{-1})	Stover N (kg ha^{-1})	Stover yield (kg ha^{-1})	Seed yield (kg ha^{-1})	Total dry matter (kg ha^{-1})	Stover N (kg ha^{-1})
Mungbean cv. T-51	3920c	1555	5475d	87.8cd	3128bc	1792ab	4920bc	64.5c
Mungbean cv. MI-5	1679d	1407	3086e	36.0d	1597c	1125b	2722c	30.2c
Cowpea cv. Bombay	5139c	1593	6732bcd	94.9c	2383c	1333ab	3716bc	47.5c
Cowpea cv. MI-35	4660c	1704	6364cd	104.5bc	2368c	1375ab	3743bc	41.5c
Cowpea cv. Arlington	6941bc	1537	8478abc	154.9b	3001bc	2083a	5084abc	59.5c
Blackgram cv. MI-1	7181b	1592	8773ab	155.2b	5179ab	1917a	7096a	104.4b
Sunhemp	9560a	–	9560a	251.7a	6124a	–	6124ab	160.9a

Within a column, values followed by the same letter are not significantly different at $P=0.05$

Table 5 Dry matter production, stover N yield, and N derived from the soil and atmosphere in the legumes, experiment 3

Crop	Total dry matter (g pot^{-1})	Stover N yield (mg pot^{-1})	Percentage of N derived from		Amount of N derived from	
			Soil (%)	Atmosphere (%)	Soil (mg pot^{-1})	Atmosphere (mg pot^{-1})
Groundnut cv. MI-1	98.9a	969.3ab	16.4c	77.8a	313.4a	1486.8a
Cowpea cv. Bombay	39.7cd	566.0c	25.7ab	65.3b	225.4b	571.9c
Blackgram cv. MI-1	55.9c	867.6b	17.8c	75.7a	213.5b	908.1b
Mungbean cv. MI-5	27.5de	355.5c	28.7a	61.2b	171.6c	366.4c
Pigeonpea ICPD 60	46.8c	1165.9a[a]	19.4bc	73.8a	225.9b	861.7b
Maize cv. Badra	73.9b	348.7c[a]		–		–
Sesame cv. MI-3	19.4e	114.0d		–		–

Within a column, values followed by the same letter are not significantly different at $P=0.05$
[a] Included all pods which were immature

Mungbeans fixed the lowest amount of N_2. The per cent N derived from the soil was lowest in groundnuts and highest in mungbeans. But, due to high levels of phytomass production, groundnuts took up the highest quantity of N from the soil.

Effect of the preceding crop on the growth and N yield of rice

Both the dry matter production and the N yield of rice were affected by the preceding crop (Table 6), and were significantly correlated with N_2 fixed by the preceeding crop. The correlation coefficient between N_2 fixed by the legumes and dry matter production by the succeeding crop was 0.459*. It was 0.619** between N_2 fixed and N yield by the succeeding crop. Compared to maize, the increase in dry matter production and N yield by rice caused by the preceding legume crop varied from about 23 to 66% and 3 to 84%, respectively, depending on the legume species. The mungbean had a significantly less beneficial effect on the succeeding rice crop than groundnuts, cowpeas and blackgram, but did not differ from sesame in this respect. The dry matter yield of rice was significantly higher when preceded by sesame than by maize. Thus, the use of crops of different families in a crop rotation appears to confer benefits on the succeeding crop which are not associated with N_2 fixation.

Discussion

Sunhemp derived more than 90% N from the atmosphere and produced the highest stover yield and N content. Thus, it was most promising as a biofertilizer for rice compared to *S. rostrata, S. sesban,* cowpeas, mungbeans, and blackgram. The high per cent N derived from the atmosphere by this crop may have been a result of the low level of available N in the soil, i.e. 0.097% of total N (Table 1). In the field study (experiment 2) sunhemp accumulated 160–250 kg N ha^{-1} within 60 days depending on location, but no information on per cent N derived from the atmosphere is available for this field study. There is a dearth of reliable estimates of the N_2 fixed by this crop. Becker et al. (1990) reported that sunhemp derived 70% N from the atmosphere, fixing 140 kg N ha^{-1} in 56 days. Assuming that 70% N was derived from the atmosphere by sunhemp in the present field study, the level of N_2 fixation was about 112–175 kg ha^{-1} in 60 days or 1.86–2.92 kg N ha^{-1} day^{-1}. The phytomass of sunhemp contained about 4% N 60 days after planting (data not given). Plant materials with an N content of about 2% or more will mineralize within a week (Jenkinson 1981). In a study involving sunhemp, sesbania, and cowpeas, a major part of the C added was mineralized in the first 2 weeks of incubation (Beri et al. 1989). Thus, establishment of rice immediately after incorporating the green manure would foster efficient use of the NH_4^+ being released during the early flush of mineralization (Beri et al. 1989). In view of the difficulties of incorporating bulky phytomass (6.0–9.5 t ha^{-1} containing 160–250 kg N in the present study) and high consequential losses of N due to denitrification and leaching, it would be better to apply the phytomass in two or three installments if suitable storage facilities are available.

In the present study, *S. rostrata* and *S. sesban*, which are green-manure crops of lowland ecosystems, showed poor adaptability and tardy growth, in agreement with previous observations (R. Senaratne and D.S. Ratnasinghe, unpublished data, 1990). However, studies in India have shown a marked superiority of *S. rostrata* over sunhemp in phytomass production and N yield (Salam et al. 1989). N contributions ranging from 90 to over 250 kg N ha^{-1} have been reported from *S. rostrata* and *S. sesban* (Rinaudo et al. 1983; Palm et al. 1988; Pareek et al. 1990). The poor growth of these *Sesbania* spp. in the present study may have been caused by establishment under upland conditions and an unfavourable day-length regime. *S. rostrata*, a short-day plant, is an annual sahelian legume that has been introduced to Sri Lanka. Experiment 1 was planted in September, and thus the plants were exposed to short-day conditions, inducing early flowering in *S. rostrata*. It has been reported from The Philippines (Watanabe and Liu 1992) that the number of days required to accumulate 100 kg N ha^{-1} in this species ranged from 41 (May planting) to 61 (December planting).

There were significant inter- and intraspecific differences in the N-supplying potential of the grain legumes, and blackgram appeared most promising in this respect. Thus, there is scope for productivity improvements in rice-based cropping systems through the identification of grain-legume species/varieties with good adaptability and high N-supplying potential in stover (Kulkarni and Pandey 1988; Buresh and De Datta 1991).

Of the five grain legumes used in experiment 3, groundnuts showed the highest N_2 fixation and mungbeans the lowest, in accord with previous observations (Senaratne and Ratnasinghe 1993). Next to the groundnut was blackgram in terms of N_2 fixation which may, at

Table 6 Effect of the preceding crop on the dry matter production and N yield of rice, experiment 3

Preceding crop	Dry matter production (g pot^{-1})	Increase in dry matter relative to maize (%)	N yield (mg pot^{-1})	Increase in N yield relative to maize (%)
Groundnut	65.8a	64.9	659.7a	83.8
Cowpea	65.9a	65.1	593.3ab	65.3
Blackgram	66.2a	65.9	540.5abc	50.5
Mungbean	49.2b	23.3	370.9de	3.3
Pigeonpea	59.2ab	48.3	489.3bcd	36.3
Maize	39.9c	–	358.9de	–
Sesame	52.6b	31.8	429.8cde	19.7
Fallow	39.8c	−0.25	301.8e	−15.1

Within a column, values followed by the same letter are not significantly different at $P = 0.05$

least partly, explain its superior performance over the other grain legumes used in experiment 2. Though the groundnut had the highest soil N uptake, it added the greatest amount of N to the soil due to its high N_2 fixation and N content in stover (Table 5). The growth and N yield of the succeeding rice crop was positively correlated with the amount of N_2 fixed by the preceding legume. This clearly shows that incorporation of legume species/cultivars with high N_2 fixation potential will add to the yield of the rice crop in sequential cropping systems.

A salient feature observed in experiment 3 was that the dry matter production of rice was significantly greater when preceded by sesame than by maize (Table 5). Maize, being a C4 plant, is more soil nutrient-exhaustive than sesame (C3). Thus, the soil nutrient status may have been higher following sesame than following maize, which may explain the above finding. Further, it has been observed that rotations with non-legume species can improve the yield as much as rotations with legumes (Langer and Randall 1981). The benficial effects of crop rotation can arise from both N and non-N effects (Welch 1977; Baldock et al. 1981; Senaratne and Hardarson 1988; Bezdicek and Granatstein 1989; Weil and Samaranayake 1991). The non-N effects have been referred to as rotation effects (Hesterman et al. 1987) and can account for as much as a 25% yield increase in the succeeding crop (Baldock et al. 1981). The rotation effect is not always present or measurable (Shrader et al. 1966; Baldock and Musgrave 1980). The sesame/rice rotation that produced a greater yield of rice than the maize/rice rotation may have demonstrated a rotation effect, the causes of which are still not well understood (Baldock et al. 1981). It is therefore important to ascertain the potential causes of rotation effects and the factors affecting their magnitude so that optimum benefits can be derived from crop rotation.

In conclusion, sunhemp, because of high N_2 fixation, good adaptability, and fast growth on paddy soils, can add well over 100 kg N ha^{-1} over 60 days. The crop is time-tested, relatively free from pests and diseases, and needs hardly any attention. Thus, it shows great promise as a biofertilizer for rice. When water is limiting for rice, the establishment of grain legumes such as blackgram and cowpeas not only produces an economic grain yield but can add considerable amounts of residual N to the soil, enhancing the productivity of rice-based cropping systems.

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